

# Studentoppgave

*Maintained number of oocytes with advanced age in PCOS women during IVF treatment compared to controls*

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## **Abstract**

PCOS is the most common reason to anovulatory infertility, and previous observations suggest that the fertility in these women increases when ageing. We wanted to investigate whether PCOS women have an increased age related ovarian response to ovulation induction, compared to patients with normal ovaries, with respect to the number of collected oocytes, clinical pregnancies and live births.

1500 patients ranging from 20-44 years of age were evaluated in this study.

By selection of patients from our clinical database at Kvinnekliviken, Rikshospitalet, we ended up with a case cohort of 500 infertile women with PCOS treated with IVF.

Two control cohorts were selected from infertile women with tubal factor infertility only and male factor infertility only. A similar distribution of age among the groups was obtained by matching, ending with 500 patients in each group.

In the PCOS group, no significant association between age and number of collected oocytes was observed. In the tubal and male factor infertility groups, advanced age was associated with reduced number of retrieved oocytes. In the PCOS and the male factor infertility groups, a significant association between age and live birth rate were not observed. In the tubal factor infertility group, advanced age was associated with a significantly reduced probability for live birth.

The results demonstrate that the ovarian capacity to release oocytes remain stable with advancing age in PCOS women, whereas it decreases in control women. It may also indicate that PCOS women do not have a decline in fertility until at least 40 years of age, whereas it decreases in normal controls.

## Introduction

The polycystic ovary syndrome is the most common endocrine and metabolic abnormality affecting 6-8% of women in their reproductive age<sup>1</sup> and the most common reason to anovulatory infertility<sup>2</sup>. It is characterized by anovulation, hyperandrogenism and polycystic ovaries (PCO) on ultrasonography. The menstrual irregularities in PCOS women are characterized by oligo- or amenorrhea with infrequent or absent ovulation leading to infertility. These women often have a normal or slightly delayed onset of menarche. In some women the menstrual irregularities do not start before they gain weight. The clinical signs of hyperandrogenism are hirsutism with thick and pigmented facial and/or body hair in a typically male pattern, acne and in some cases male pattern hair-loss.

In addition, some PCOS women have dyslipidemia<sup>3</sup>, 9-17% the metabolic syndrome<sup>4</sup>, 50-70% insulin resistance<sup>5</sup> and thus an increased risk of developing type 2 diabetes mellitus<sup>6</sup>, and possibly cardiovascular diseases<sup>7</sup>.

The mechanisms responsible for the elevated androgen levels is thought to be caused by an increased production and secretion of androgens by hyperplastic theca cells<sup>8</sup>. A possible increase in pulsatile release of gonadotrophin releasing hormone (GnRH) from the hypothalamus leads to an increased release of luteinising hormone (LH) from the pituitary gland. The cause of the syndrome remains uncertain, but it is most likely linked to genetic factors<sup>9</sup> with abnormal follicle growth and maturation<sup>10</sup>.

Generally, fewer follicles grow per cycle and pregnancy rates decline with advancing age<sup>11, 12</sup> as oocyte quantity and quality will rapidly decline in the late thirties<sup>13</sup>. Thus, about 1/3 of women at 35 and 1/2 of normal women at 41 years of age are infertile<sup>14</sup>. PCOS patients have an enlarged follicle cohort<sup>15</sup>, and have significantly more oocytes collected in in vitro maturation programs compared to normal controls<sup>16</sup>. However, on ultrasound examination of the ovaries, Bili et al found that older PCOS women present a reduced number of ovarian follicles in response to gonadotropin stimulation compared to younger PCOS women<sup>17</sup>.

PCOS women are born with an increased population of germ cells or have a decreased rate of oocyte loss until puberty<sup>15</sup>.

Thus, the aim of this study was to investigate whether PCOS women have an increased age related ovarian response to ovulation induction, compared to patients with normal ovaries, with respect to the number of collected oocytes, clinical pregnancies and live births.

## **Methods**

In this section, I will make a brief summary of the most important considerations that were made in our study, based on the work of Peter Fedorcsák and Jan Mellembakken.

### ***Selection of patients***

All patients used for this case-control study were retrospectively identified from a clinical database at the Department of Obstetrics and Gynecology, Rikshospitalet. Approval from database query was obtained from the “Ombudsmann for Privacy and Data Protection”.

The PCOS group consisted of patients with at least two of the following three conditions present: oligomenorrhea or amenorrhea, hyperandrogenism or hirsutism and polycystic ovaries (Rotterdam criteria, 2003<sup>18</sup>). Patients with NCAH (non-classical congenital adrenal hyperplasia), Cushing’s syndrome, androgensecreting tumours and hyperprolactinemia were excluded by appropriate tests. Patients with co-existing diseases, including tubal disease, male factor infertility and endometriosis were excluded by a thorough review of clinical records. In the end our case cohort contained 500 PCOS women from 20-44 years of age.

We decided to have two control groups. 500 women who were treated with IVF or ICSI for tubal factor infertility and 500 women with male factor infertility were selected by matching each woman in the PCOS group to the control groups by age. Women with other co-existing conditions, including endometriosis, PCOS, as well as history of ovarian surgery were excluded from the control groups.

### ***Ovarian stimulation and fertilization in vitro***

In vitro fertilization (IVF) is a procedure where the ovaries are stimulated and oocytes are collected from the ovarian follicles to be fertilized in vitro and eventually one or two embryos are being transferred back to the uterus.

Before this IVF cycle, both partners should have a complete infertility evaluation.

In PCOS women, IVF is performed when other treatments like weight loss and ovulation induction with clomiphene citrate show no effect on fertility.

Intracytoplasmic sperminjection (ICSI) is a technique in which a single sperm is injected directly into the cytoplasm of a mature oocyte to treat male factor infertility.

In some cases, the use of gonadotrophins for ovulation induction may cause complications. One concern is the increased incidence of multiple pregnancies with an increased risk of perinatal mortality and morbidity. Another is the ovarian hyperstimulation syndrome (OHSS), that may occur during excessive ovarian stimulation and is a potentially life-threatening complication to ovulation induction.

All patients had an ultrasound examination of the ovaries before being treated with IVF. The age, weight and presence of PCO or not, decided the FSH dose given to the patient.

Pituitary down regulation was achieved with GnRH agonist administered intranasally from the mid-luteal phase of the preceding cycle. Recombinant FSH or human menopausal gonadotrophin was given for ovarian stimulation. The standard starting dose was 75 IU daily in the PCOS group and 150 IU daily in the control groups. Women older than 35 received 200-225 IU daily FSH as starting dose. The dose was adjusted after 4-9 days according to the ovarian response<sup>19</sup>.

Collected oocytes were fertilized with ejaculated spermatozoa in vitro by IVF or ICSI, using standard procedures<sup>19</sup>. In order to reduce the effect of dose adjustment based on prior treatment experiment, only the first treatment of patients with multiple treatment cycles was considered. Pregnancy was defined by serum hCG concentration > 20 IU/l on day 16 after embryo transfer.

OHSS was diagnosed by combination of clinical symptoms as abdominal distension, nausea, excessive follicle development or ascites. Body mass index (BMI, kg/m<sup>2</sup>) was assessed at the first visit using the same weight scale and height stock.

### ***Data analysis***

Data are shown as mean and standard deviation or median and range. Findings was considered statistically significant at  $P < 0.05$ .

The association between the number of collected oocytes and age was assessed by using multiple regression analysis, after controlling for mean daily FSH dose and BMI. T-test was used to find the regression coefficients (slopes) for age comparing the

groups. To find any association between birth rate and age, logistic regression analysis was used after controlling for the number of collected oocytes.

## ***Literature***

The search for literature has been directed to find related articles for the study and to find articles and reviews to learn more about the subject of PCOS, by using MedLine/Pubmed. My mentor also provided some articles relating to our study. Searchword like PCOS and age/ageing/IVF/oocytes/fertility/infertility was used to narrow the searchfield. In some cases articles was found by using the references in other articles to search by names, title and in some cases year/date of publishing. UpToDate.com and teaching textbooks in gynecology provided much background information on PCOS. Topics here were clinical manifestation, epidemiology, pathology, diagnosis and treatment.

## **How I assessed the articles found in the search**

- Is it clear in the introduction which population is being studied and the intervention given?
- Is the study randomised and is it the best way of answering the question? Is it a good enough randomisation procedure (blinded etc)?
- If two groups are compared, are they comparable? Are the groups equal enough? (Selection bias? –confounding variables? Observation bias? )
- Did the groups get the same treatment?
- Is there a statistical significant difference between the groups?
- Could the results just be a coincidence? –what is the confidens interval and p-value?



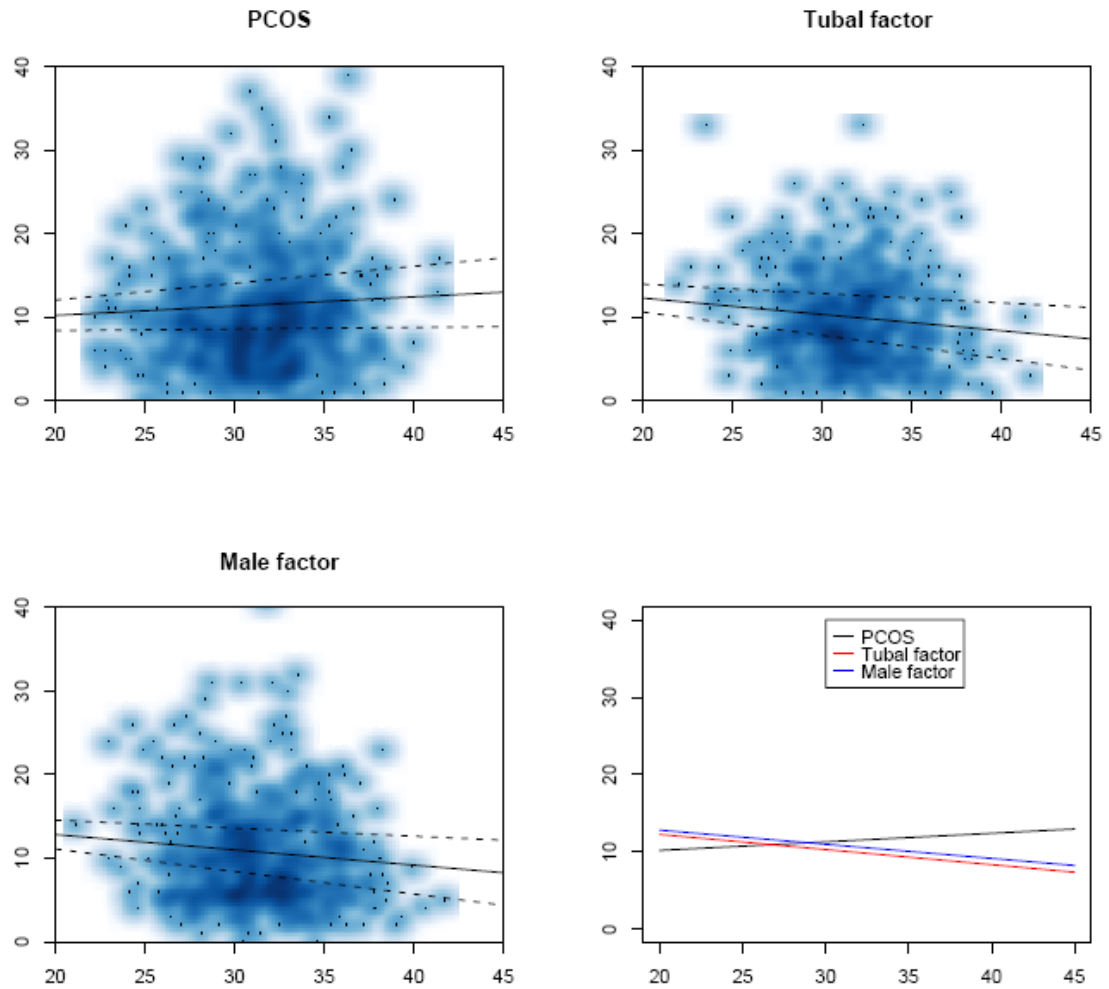
## Results

	PCOS (n=500)	Tubal factor (n=500)	Male infertility (n=500)	P-value
Age (years)	31.3 (3.6)	31.6 (3.2)	31.3 (3.3)	
BMI (kg/m <sup>2</sup> )	26.5 (5.2)	23.5 (3.9)	23.9 (4.0)	<0.001
Total FSH-dose (IU, median range)	1650 (525-9825)	1725 (525-2500)	1650 (450-11675)	0.08
Duration of FSH treatment (days)	12 (6-39)	11 (6-28)	10 (5-19)	<0.001
No. of oocytes collected	9 (0-39)	9 (0-33)	10 (0-41)	0.19
No. of diploid fertilized oocytes	5 (0-25)	6 (0-23)	4 (0-20)	<0.001
No. of transferred embryos	2 (0-3)	2 (0-3)	2 (0-3)	
Pregnancy rate (hcg +)	169 (33.8%)	169 (33.8%)	168 (33.6%)	0.99
Pregnancy loss	47 (27.8%)	36(21.3%)	37 (21.4%)	0.28
No. of live births	121 (24.2%?)	124 (24.8%?)	125 (25.0%?)	0.954
Ovarian hyperstimulation syndrome	11 (2.2%)	3 (0.6%)	1 (0.2%)	0.004

**Table 1**

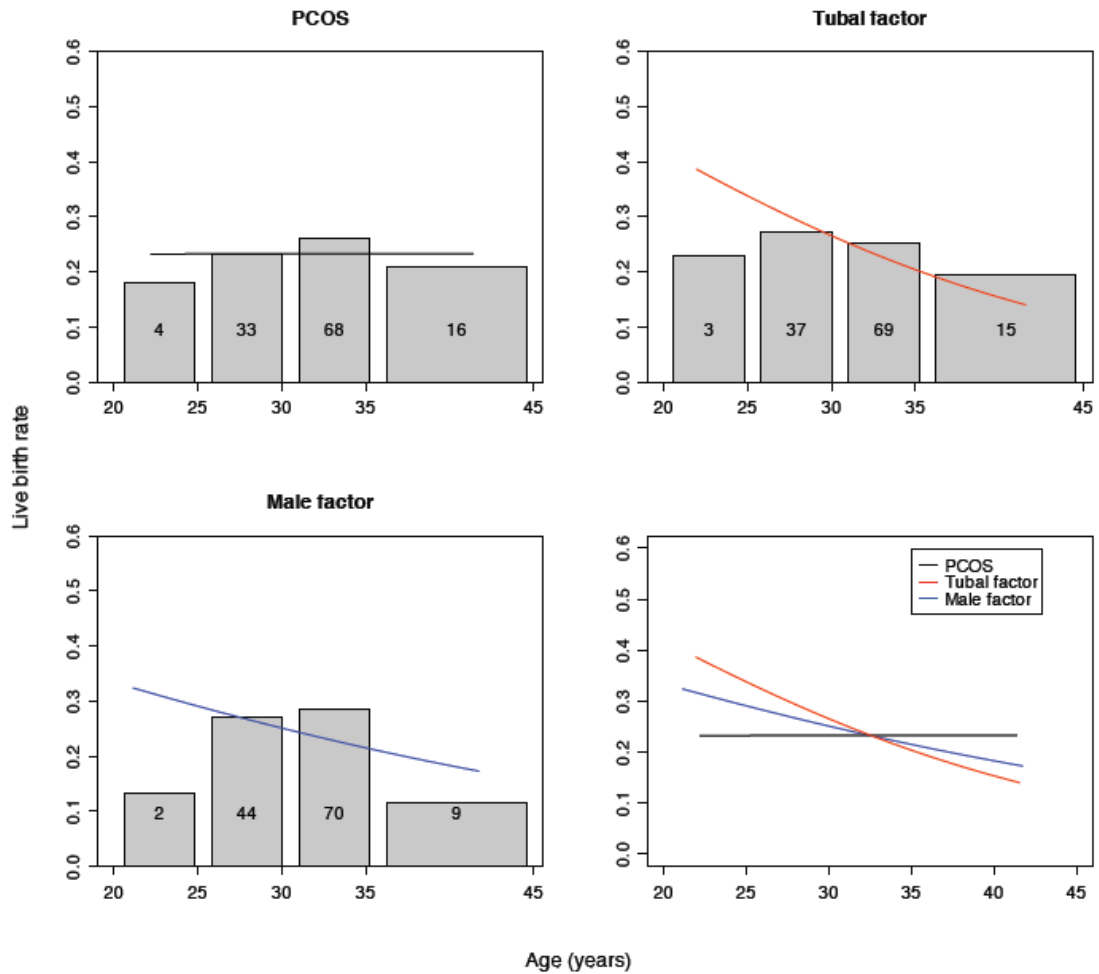
Clinical characteristics, ovarian stimulation and treatment outcome among age matched women with PCOS, tubal factor infertility and male factor infertility undergoing assisted reproduction treatment.

The mean body mass index (BMI) was, as expected, significantly higher in the PCOS group than the control groups ( $P < 0.001$ , Table 1). Statistically there was no difference among the groups in the number of oocytes collected. But when using multiple regression analyses with age, mean daily FSH doses and BMI as variables it shows that in the PCOS group there is no association between age and the number of collected oocytes. In both the tubal factor infertility group and male factor infertility group advanced age was associated with a reduced number of retrieved oocytes (Figure 1). The duration of FSH treatment is significantly longer for PCOS women, but the total FSH dose given is the same between the groups. The number of fertilized oocytes is higher ( $p < 0.001$ ) in the tubal factor group compared to the PCOS group, but not compared to the male factor infertility group. There was no statistical difference in pregnancy rate and pregnancy outcome between the groups, but age-related changes are shown when using logistic regression analysis. In the tubal factor infertility group, advanced age was associated with a significantly reduced probability for live birth. This was not shown in the PCOS and male factor infertility group (Figure 2). The prevalence of ovarian hyperstimulation syndrome (OHSS) was as expected higher in the PCOS group compared to the control groups (Table 1).



**Figure 1.**

Age-related changes in the number of oocytes collected after ovarian stimulation with FSH in age-matched women treated for PCOS, tubal factor infertility and male factor infertility. The observed data were plotted using smoothed color density representation for the number of observations. Fitted solid lines indicate the association between age and the number of collected oocytes, and were derived from multiple regression analysis using age, mean daily FSH dose, and BMI as covariates.



**Figure 2.**

Age-related changes in live birth rate after assisted reproduction treatment among age-matched women treated for PCOS, tubal factor infertility and male factor infertility. Bars indicate observed live birth rate in the given age categories with the number of live births enclosed in the bars. Fitted lines indicate the association between age and live birth rate, and were derived from logistic regression analysis using age and the number of collected oocytes as covariates.

## Discussion

The results in our study shows that PCOS women's capacity to release oocytes during IVF treatment does not decline and remains stable with increasing age up to <45 years, whereas it decreases in the control groups. In addition, we found no association between age and live birth rate in PCOS women.

In the tubal factor infertility group the fertility decreased whereas in the male factor group the decrease with age was not significant. However, it is possible this would have reached a statistically significant level with a larger control group.

Our results are in contrast to Guido et al who found an age related decline in the response to a low-dose gonadotrophin ovulation induction treatment in PCOS patients<sup>20</sup>. One reason for the difference in findings may be that Guido et al did use a low-dose regime, in order to induce unifollicular ovulation in PCOS patients. In in vitro maturation programs, where patients are not given FSH stimulation, PCOS patients have an increased number of oocytes collected compared to controls<sup>21</sup>.

The duration of FSH treatment is in our study significantly longer for PCOS women, but the total FSH dose given is the same between the groups. Because of the risk of OHSS, ovarian hyperstimulation syndrome, PCOS women receive a lower starting dose, in order to prevent this complication. The starting dose of FSH is about half the dose of other IVF-treated women<sup>19</sup>. It has been presumed, that PCOS women need a smaller dose of FSH for oocyte maturation, but the results of this study indicate that they may need the same amount of FSH as normal controls to achieve oocyte maturation for IVF.

Ovarian ageing is an irreversible process. The number of follicles declines with age, faster after the age of 37.5, leading to the menopause at around 51 years of age<sup>22, 23</sup>. Secondary to the physiological ovarian failure, FSH begin to increase in the early follicular phase at the age of 35<sup>24</sup>. The LH secretion changes around the age of 45<sup>23</sup>. In normo-ovulatory women, the Anti-Müllerian hormone (AMH) levels decline with age<sup>25</sup>. These levels are correlated to the number of antral follicles with AMH having the best correlation<sup>26</sup>.

Polycystic ovaries in young women have twice the number of primary follicles compared to normal age-matched ovaries<sup>17, 27-29</sup> and up to a sixfold increase in the number of primordial and primary follicles in anovulatory women with PCOS compared to normal regularly cycling women<sup>15</sup>. A high number of follicles producing an increased level of androgens contributing to menstrual irregularities and anovulation clinically and possibly contribute to the lower number of oocytes recruited from PCOS women before 28 years of age, compared to controls (figure 1). However, it may also reflect that PCOS women may have a slower growth through pre-antral stages compared to controls<sup>15</sup>.

The influence of age on fertility of PCOS women, have seldom been discussed in the literature, but has been presumed to impair treatment outcome<sup>30</sup>. A decrease in the follicular pool is also present in ageing PCOS women<sup>17</sup>. But the decrease in inhibin B, that follows the follicular loss<sup>24, 31</sup>, may facilitate more regular and more frequent ovulatory cycles in these women. Because anovulatory PCOS women have a larger follicular pool, they are unlikely to undergo a rapid depletion of their ovarian reserve<sup>13</sup>. This is supported by that the decrease in AMH when ageing is less obvious in PCOS women, possibly contributing to a longer reproductive life compared to controls<sup>21</sup>. PCOS ovaries may contain both an increased number of oocytes and release them slower, ending up with an increased number of oocytes and sustained fertility later in life. This is supported by the clinical observation by Vulpoi et al. that spontaneous pregnancies did occur late in life in three previously infertile PCOS women<sup>32</sup>. Because these women became pregnant years after their last infertility treatment, no hormonal tests and clinical evaluations were done prior to the spontaneous pregnancies, and it should have been a larger study investigating PCOS women over time to see if this increase in fertility is significant.

In conclusion, as PCOS women may possess a higher number of oocytes throughout fertile years and obtain a more favourable inhibin B level late in life, they may have an increased release of oocytes and unchanged fertility with advancing age compared to controls. Regarding the fertility, our study conclude that PCOS women does not have a decline in fertility with increasing age up to 40 years of age.

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